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14. ABSTRACT
E. coli mazEF is a stress-induced toxin-antitoxin system discovered by us as being responsible for Programmed Cell Death (PCD) in the bacteria. Recently, we showed that under condition of sever DNA damage, the triggered mazEF-mediated death pathway leads to the inhibition of an Apoptotic-Like Death (ALD) pathway mediated by recA and lexA. The well known SOS pathway is an additional cellular response to DNA damage mediated by recA-lexA. It is the largest, most complex, and best characterized bacterial network induced by DNA damage

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Report Title

Bacterial Programmed Cell Death as a Population Phenomenon

ABSTRACT

E. coli mazEF is a stress-induced toxin-antitoxin system discovered by us as being responsible for Programmed Cell Death (PCD) in the bacteria. Recently, we showed that under condition of sever DNA damage, the triggered mazEF-mediated death pathway leads to the inhibition of an Apoptotic-Like Death (ALD) pathway mediated by recA and lexA. The well known SOS pathway is an additional cellular response to DNA damage mediated by recA-lexA. It is the largest, most complex, and best characterized bacterial network induced by DNA damage Therefore, here we asked whether the mazEF-mediated pathway also inhibits the SOS response. We found that indeed this is the case. Under mild DNA damage, the expression of mazEF inhibits the SOS response. We examined various E. coli strains commonly used for studies of the SOS response. We found that SOS response only took place in E. coli cells in which one or more elements of the E. coli toxin-antitoxin module mazEF was not functioning. Thus, the interplay between the SOS response and the mazEF mediated pathway broaden the degree of the bacterial response to DNA damage. Our work reflects the complexity of the interplays between cellular networks, and as such reflects the importance of personalized medicine.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
03/22/2012	4.00	Ariel Erental, Idith Sharon , Hanna Engelberg-Kulka. Two Programmed Cell Death Systems in Escherichia coli:An Apoptotic-Like Death Is Inhibited by the mazEFMediatedDeath Pathway, PLoS Biology, (03 2012): 0. doi:
03/30/2011	2.00	Finbarr Hayes. Moving in for the kil:Activation of an endoribonuclease toxin by quorum sensing peptide, Molecular Cell, (03 2011): . doi:
06/11/2013	11.00	Isabella Moll, Hanna Engelberg-Kulka. Selective translation during stress inEscherichia coli, Trends in Biochemical Sciences, (11 2012): 493. doi:
07/19/2012	6.00	Oliver Vesper,, Shahar Amitai,, Maria Belitsky,, Konstantin Byrgazov,, Anna Chao Kaberdina,, Hanna Engelberg-Kulka,,* and , Isabella Moll,*. Selective Translation of LeaderlessmRNAs by Specialized RibosomesGenerated by MazF in Escherichia coli, Cell, (09 2011): 0. doi:
07/19/2012	5.00	Maria Belitsky,, Haim Avshalom,, Ariel Erental,, Idan Yelin,, Sathish Kumar,, Nir London,, Michal Sperber,, Ora Schueler-Furman, and , Hanna Engelberg-Kulka,*. The Escherichia coli Extracellular Death FactorEDF Induces the Endoribonucleolytic Activitiesof the Toxins MazF and ChpBK, Molecular Cell, (03 2011): 0. doi:
07/19/2012	8.00	Richard Robinson*. In E. coli, Interrupting One Death Pathway Leads YouDown Another, PLoS Biology, (03 2012): 0. doi:
07/19/2012	9.00	Andrew Jermy. Stressed bacteria aren't lost without a leader, Nature Reviews, (11 2011): 0. doi:
TOTAL:	**7**	

Number of Papers published in peer-reviewed journals:

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Received Paper

TOTAL:

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(c) Presentations

1. A lecture in the meeting on Structural and Functional diversity of Genomes. 2nd International Mendel Workshop. Brno, Czech Republic. 2012.
The lecture: Prof. Hanna Engelberg-Kulka,
2. A lecture in the graduate course in Karolinske University, Stockholm, Sweden. 2012.
The lecture: Prof. Hanna Engelberg-Kulka,
3. A lecture in Seminar Workshop in Microbial Biology, Hydrabard, India. 2012.
The lecture: Prof. Hanna Engelberg-Kulka,
4. A lecture in Developmental Biology of Microbial Biofilms. NTU, Singapore. 2013.
The lecture: Prof. Hanna Engelberg-Kulka,
5. A lecture in the Gordon Research Conference on Mechanisms of Biological Evolution. 2013.
The lecture: Prof. Hanna Engelberg-Kulka,

Number of Presentations: 5.00

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(d) Manuscripts

Received		Paper
06/11/2013	10.00	Isabella Moll, Hanna Engelberg-Kulka. Selective translation during stress in Escherichia coli, Trends in Biochemical Sciences (11 2012)
08/04/2011	3.00	Oliver Vesper, Shahar Amitai, Maria Belitsky, Konstantin Byrgazov , Anna Chao Kaberdina, Hanna Engelberg-Kulka, and Isabella Moll. Specialized Ribosomes Generated by MazFSelectively Translate Leaderless mRNAsUpon Stress in Escherichia coli, Cell (08 2011)
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Books

Received	Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Ariel Erental	1.00	
Sathish Kumar	1.00	
FTE Equivalent:	**2.00**	
Total Number:	**2**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
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The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Ziva Kalderon
Total Number: **1**

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT SUPPORTED

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Introduction

E. coli mazEF is a toxin -antitoxin system that was discovered by us as being responsible for Programmed Cell Death (PCD)(1), and is since extensively studied by us (2-4) and by others (5). We have also shown that E. coli mazEF-mediated cell death is a population phenomenon requiring the E. coli quorum sensing factor EDF (Extracellular Death Factor) (6-7). Structural analysis revealed that EDF is the linear penta-peptide Asn-Asn-Trp-Asn-Asn, required for triggering mazEF-mediated cell death (6). The toxin MazF is a sequence-specific endoribonuclease that preferentially cleaves single-stranded mRNAs at ACA sequences (8,9). We have shown that EDF amplifies the endoribonucleolytic activity of MazF (10). As previously reported (8), MazF induction causes inhibition of protein synthesis. However, we have reported that surprisingly this inhibition was not complete: though MazF led to the inhibition of the synthesis of most proteins (about 90%), it selectively enables the specific synthesis of about 10% of proteins (3). Some of those proteins were required for the death of most of the population. We have recently elucidated the molecular mechanism responsible for the selective synthesis of these proteins. We found that: a) MazF cleaves at ACA sites at or closely upstream to AUG-start codons of specific mRNAs, and thereby generating leaderless mRNAs belonging to a novel “Leaderless regulon”; and b) MazF targets the 16S rRNA within the 30S ribosomal subunit at the decoding centre, thereby removing 43 nucleotides from the 3’-terminus. Since these 43 nucleotides include the anti-SD (Shine-Dalgarno) region, these deficient ribosomes, that we call “stress ribosomes”, are selectively able to translate the generated leaderless mRNAs (4). Thus, under stressful conditions, MazF is induced, which leads to the generation of a novel “leaderless regulon” that is translated by the novel “stress ribosomes”, producing a distinct pool of “stress proteins” Some of these proteins leads to the death of the bacterial population.

Recently, using confocal microscopy and FACS analysis we showed that under condition of sever DNA damage, the triggered EDF-mazEF-mediated cell death pathway leads to the inhibition of a second cell death pathway. The latter is an Apoptotic-Like Death that we have called ALD; ALD is mediated by recA and lexA (10). The well known, extensively studied SOS pathway (reviewed by 11-15) is also a cellular response to DNA damage, and is also mediated by recA-lexA. In an uninduced cell, the lexA gene product, LexA, acts as a repressor of more than 40 genes (16-17), including recA and lexA, by binding to operator sequences (called SOS box) upstream to each gene or operon. Under conditions of DNA damage, regions of single-stranded DNA are generated that convert RecA to an active form that facilitate an otherwise latent capacity of LexA (and some other proteins like UmuD and the □CI repressor) to autodigest (11-12, 14-16, 18). Here we asked: Does the E. coli EDF-mazEF pathway inhibit the SOS bacterial response? The mazEF pathway is present on the chromosomes of most E. coli strains (19,20). Therefore, If the EDF-mazEF pathway inhibits the SOS response, why is the SOS response found in so many E. coli strains? Perhaps the EDF-mazEF pathway is present but inactivated in those strains?

Specific Aims

We undertook the following main directions:

1)We asked : Does the E. coli EDF-mazEF pathway inhibit the SOS bacterial response?

2)The mazEF pathway is present on the chromosomes of most E. coli strains (19,20). Therefore, If the EDF-mazEF pathway inhibits the SOS response, why is the SOS response found in so many E. coli strains? Perhaps the EDF-mazEF pathway is present but inactivated in those strains.

Results

In E. coli strain MC4100relA+, the SOS response is prevented by the mazEF module and by some genes downstream from mazEF .

To study the effect(s) of the mazEF mediated pathway on the SOS response, we used plasmid pL(lexO)-gfp (21), which bears gfp, the gene for the green fluorescent protein (GFP), under the control of the lexA operator, lexO. In this system, under uninduced conditions, LexA represses gfp transcription by binding to the SOS box in the gene operator, lexO. Under DNA damage, RecA becomes activated, and acts as a co-protease stimulating the inactivation of LexA by auto-cleavage. Thereby the gfp gene can be transcribed, and its fluorescence can be detected. Thus, in this system, fluorescence is a reporter for the RecA dependent SOS response. Using this fluorescence reporter system, we caused DNA damage by adding nalidixic acid (NA) (10μg/ml) to the cultures (22). Our experiments have revealed that the SOS response was only permitted in E. coli strain MC4100relA+ in which the mazEF genes have been deleted, and not in its WT MC4100relA+ (Fig. 1A). Thus, our results suggest that mazEF may prevent the SOS response.

Previously, we reported that the induction of the mazEF mediated-death pathway activates the selective synthesis of two

groups of proteins: the products of genes yfbU, slyD, yfiD, clpP, ygcR, that participate in the death process (the “death genes”), and the products of genes elaC and deoC that lead to cell survival (the “survival genes”) (2). Here, we found that deleting the “death genes” allowed the SOS response to take place (Fig. 1B-F); however deleting the “survival genes” did not (Fig.S2). These results support the hypothesis that the SOS response cannot take place in the presence of mazEF-mediated death pathway.

The Extra-Cellular Death Factor (EDF) is involved in the inhibition of the SOS response.
Since, in previous work, we showed that EDF, the penta-peptide NNWNN, is involved in EDF-mazEF mediated death (6), and here we found that the action of mazEF module prevented the SOS response (Fig. 1), we asked if, in addition to the mazEF module, the presence of EDF is also involved in the inhibition of the SOS response. We have previously demonstrated that clpX is required for the production of EDF (7). Here we found that the SOS response was permitted not only in an E. coli MC4100relA+ strain from which we deleted mazEF (MC4100relA+ΔmazEF) (Fig 1A), but also when, instead of deleting mazEF, we deleted clpX (MC4100relA+ΔclpX) (Fig. 2A). This effect seems to be due to the lack of EDF because: (a) the addition of EDF partially inhibits the studied SOS response (by 30%), and (b) the SOS response is not affected at all by the addition of iEDF (Fig.2A), the penta-peptide NNGNN, in which the central and crucial tryptophan has been replaced by glycine (19). Adding iEDF to the MC4100relA+ΔclpX culture did not affect the SOS response at all (Fig. 2A). An additional support that EDF is involved in the mazEF mediated inhibition of the SOS response is derived from our studies with E. coli strain MG1655. In our previous work, we showed that E. coli strain MG1655, which carries the mazEF gene pair, is defective in the production of and the response to EDF (8). Here we found that, despite the presence of mazEF, the SOS response took place in strain MG1655 (Fig. 2B). Furthermore, 240 minutes after adding EDF, we observed a 50% reduction in the SOS response; in contrast, adding iEDF did not cause any reduction in the SOS response (Fig. 2B). All of these results support our hypothesis that {the SOS response was permitted in the absence of EDF.
Using our fluorescence reporter system, we tested the SOS response in four additional E. coli strains. In strains AB1157, AB1932, and SS996, the addition of EDF did not inhibit the SOS response (data not shown). However, in E. coli strain BW25113, which has commonly been used to study the phenomena of the SOS response (23-24), we were surprised to observe that the addition of EDF did prevent the SOS response (Fig. 2C). Adding EDF to E. coli strain BW25113 led to a 60% reduction in the SOS response; again, as in the case for strains MC4100relA+ clpX (Fig 2A) MG1655(Fig 2B), adding iEDF did not lead to a reduction in the SOS response (Fig.2C).

The stringent response, known to activate the mazEF-mediated death pathway, inhibited the SOS response.
Previously we found that the nutritional starvation signal ppGpp, responsible for the stringent response (25), is involved in the mazEF-mediated cell death (1). Here we asked: Is the SOS response permitted in E.coli strains defective in ppGpp production. To this end we first used E.coli strain MC4100relA1 in which the relA gene is inactivated by an insertion (relA1) (26). Indeed, we did observe the SOS response in MC4100relA1 strain. The plasmid pZA31-relA bears an anhydrotetracycline-inducible relA gene. When this relA1 strain harbored pZA31-relA, and when we added anhydrotetracycline (aTc) to the culture to induce relA, we observed no SOS response (Fig.3A). These results suggested that probably additional E. coli strains, commonly used in studies of the SOS response were defective in ppGpp synthesis. Here, we studied E. coli strains AB1157, AB1932, BW25113, MG1655, and SS996 for ppGpp production by their ability to grow in M9 plates containing 3-amino-1,2,4-triazole (AT). AT is a competitive inhibitor of imidazoleglycerol-phosphate dehydratase, a key enzyme for histidine production, and thereby causing histidine starvation leading for the production of ppGpp (27). Therefore, the ability to grow in the presence of AT is provides an assay for ppGpp production (27). We found that among these five strains, AB1157 and SS996 did not grow in the presence of AT, indicating that they were defective in (p)ppGpp production (data not shown). Note that, as we have shown here (Fig. 3B and Fig. 3C) and as has been previously shown by others (18, 28-30), the SOS response was permitted in both E. coli strains AB1157 and SS996. Moreover, as we found for MC4100relA1 (Fig. 3A), when these strains harbored plasmid pZA31-relA and in the presence of aTc, we observed no the SOS response (Fig. 3B and Fig.3C). These results support the idea that, in E. coli strains AB1157 and SS996, a defect in (p)ppGpp production, and thereby in the expression of the EDF-mazEF mediated-death pathway, allowed the SOS response to take place.

The SOS response is permitted in E. coli strains carrying prophage lambda. One of the few genes expressed by phage λ in its lysogenic state is λrexB (31-33). In previous work, we showed that its product, λRexB, inhibits the degradation of the antitoxic labile compound, MazE, thereby preventing mazF mediated death pathway (34). Therefore, we anticipated that, in contrast to E. coli strain MC4100relA+ in which the SOS response is prevented (Fig.1A), in the presence of a λ prophage the SOS response would be permitted in this strain. As we expected, the presence of the λ prophage overcame the inhibitory effect of mazEF on the SOS response (Fig. 4A). Furthermore, in this strain, when we deleted rexB from the prophage λ, the SOS response was no longer observed (Fig. 4A). As in the case of E. coli strain MC4100relA+ , we did the same experiment with E. coli strain AB1932, in which the SOS response has been observed, and which has been reported to bear a λ prophage on its chromosome (29). We observed the SOS response in strain AB1932 (Fig.4B). Deleting rexB from its λ prophage prevented the SOS response, while introducing a plasmid bearing a λ prophage and inducing for rexB permitted the SOS response (Fig.4B). Thus, our results provide an explanation for the SOS response in strain AB1932.

Conclusion

The Escherichia coli (E. coli) SOS response is the largest, most complex, and best characterized bacterial network induced by DNA damage. It is controlled by a complex network involving the RecA and LexA proteins. Here we have shown that the SOS response to DNA damage is inhibited by various elements involved in the expression of the E. coli toxin-antitoxin module mazEF. We examined various E. coli strains commonly used for studies of the SOS response, including strains AB1157, AB1932, BW25113, SS996, MG1655, and MC4100relA1. We found that each of these strains is either missing or inhibiting one of several elements involved in the expression of the mazEF-mediated death pathway. Thus, the SOS response only took place in E. coli cells in which one or more elements of the E. coli toxin-antitoxin module mazEF was not functioning. Based on these results, we suggest that the interplay between the SOS response and the mazEF mediated death pathway broaden the degree of the bacterial response to DNA damage and thereby to bacterial survival. Our work on the SOS response to DNA damage in E. coli, reflects the complexity of the interplays between cellular networks, and as such reflects the importance of personalized medicine.These results have been recently submitted for publication (35)

MAJOR ACCOMPLISHMENTS

The Escherichia coli (E. coli) SOS response is the largest, most complex, and best characterized bacterial network induced by DNA damage. During the fourth year of our research we made an exciting discovery in this well studied central bacterial cellular response. We have shown for the first time that the EDF-mazEF mediated death pathway inhibits the SOS response. Our herein results on, the SOS response to DNA damage in E. coli, reflects the complexity of the interplay between cellular networks, and as such reflects the importance of personalized medicine in general, and specifically in the use of antibiotics due to the expected diversity of individual microbiota.

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Figure legends

Figure 1. The effects on the SOS response system of the mazEF module and genes downstream from mazEF. We used E. coli MC4100relA+ and its derivatives ΔmazEF (A),ΔyfbU(B),ΔslyD(C),ΔyfiD(D),ΔclpP(E), and ΔygcR (F). We grew the cells, all of which harbored plasmid pL(lexO)-gfp in supplemented M9 media, with shaking, at 37°C, to O.D.600 0.5-0.6, and treated (or not) with NA (10μg/ml). We measured fluorescence (FU) by fluorometer over a period of 4 hours. All of the values shown are relative to those of cells not treated with NA.

Figure 2. The inhibition of the SOS response by the mazEF pathway required the participation of EDF. We used E. coli strains MC4100relA+ with MC4100relA+ΔclpX (A), MG1655 (B), or BW25113 (C); all the strains harbored plasmid pL(lexO)-gfp. We grew the cells as described in the legend to Fig. 1. When the culture reached O.D.600 0.5-0.6, we added (or not) EDF (10ng/ml) or iEDF (100ng/ml). These cultures were incubated without shaking at 37°C for 30 min, after which we added NA (20μg/ml) to each sample. Immediately after adding NA, we measured fluorescence (FU) by fluorometer over a period of 4 hours. The values shown are relative to those of cells that had not been treated with NA.

Figure 3. The SOS response is prevented in E. coli strains bearing a functional relA+ gene. We used E. coli strain MC4100relA+ and strains MC4100relA1, MC4100relA1/pZA31-relA(A);AB1157,AB1157/pZA31-relA(B) and SS996, SS996/pZA31-relA(C). All of the strains harbored plasmid pL(lexO)-gfp. The cells were grown as described in the legend to Fig. 1. At O.D.600 0.5-0.6, we added NA (10μg/ml). The strains harboring plasmid pZA31-relA were induced by the addition of aTc (10mM), and incubated without shaking at 37°C for 30 min after which we added either NA (10μg/ml) or NA (10μg/ml) plus SH (2.5 mg/ml). We measured fluorescence (FU) by fluorometer over a period of 4 hours. The values shown are relative to those of cells not treated by NA.

Figure 4. λ Lysogens overcome the inhibitory effect of mazEF on the SOS response. We used E. coli MC4100relA+ as a control strain, and two experimental parent strains lysogenized by phage λ: MC4100relA+λ+, MC4100relA+λΔrexB, and MC4100relA+λΔrexB/pZA31-rexB(A); and AB1932λ+, AB1932λΔrexB, AB1932λΔrexB/pZA31-rexB(B). All of the strains harbored plasmid pL(lexO)-gfp. Cells were grown as described in the Legend to Fig. 1 and treated (or not) with aTc. After 30 min, we added NA (10μg/ml), and measured fluorescence (FU) by fluorometer over a period of 4 hours. The values shown are relative to cells not treated with NA.

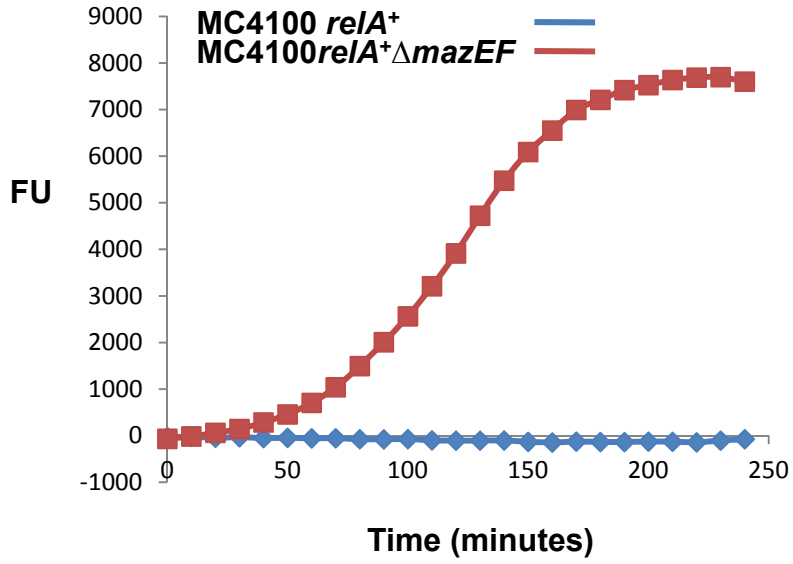
Table 1. The identified elements related to the EDF-mazEF pathway that permitted the SOS response in commonly used E. coli strains. In each of the E. coli strains in which the SOS response is commonly studied, there was one element that inactivated mazEF, and therefore was responsible for the activity of the SOS response. The circles indicate the element that was missing (-) or present (+) that permitted the SOS response in each of these strains.

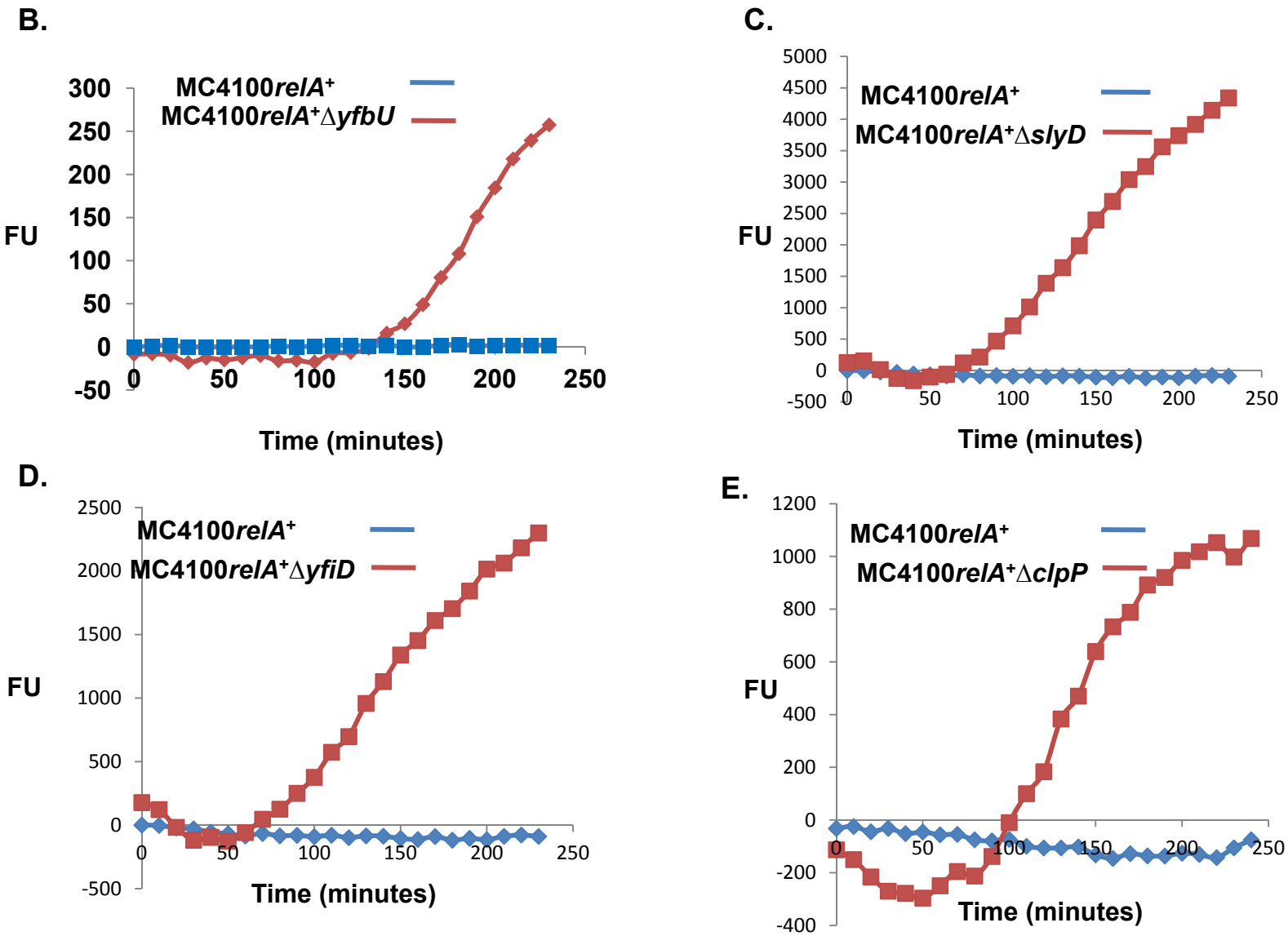
Technology Transfer

Figure 1

The effect of the *mazEF* module (A) and its downstream genes (B) on the SOS response.

A.




B.
MC4100relA+
MC4100relA+ΔyfbU
FU
Time (minutes)
C.
MC4100relA+
MC4100relA+ΔslyD
FU
Time (minutes)
D.
MC4100relA+
MC4100relA+ΔyfiD
FU
Time (minutes)
E.
MC4100relA+
MC4100relA+ΔclpP
FU
Time (minutes)

F.

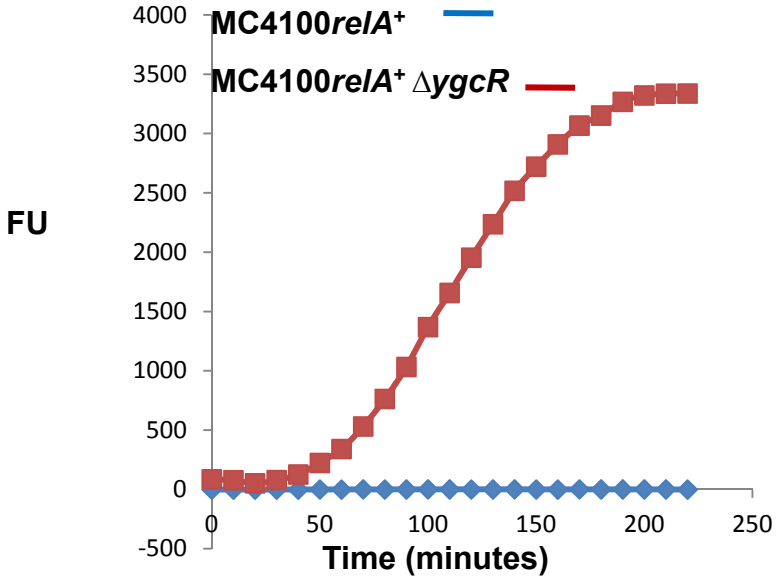
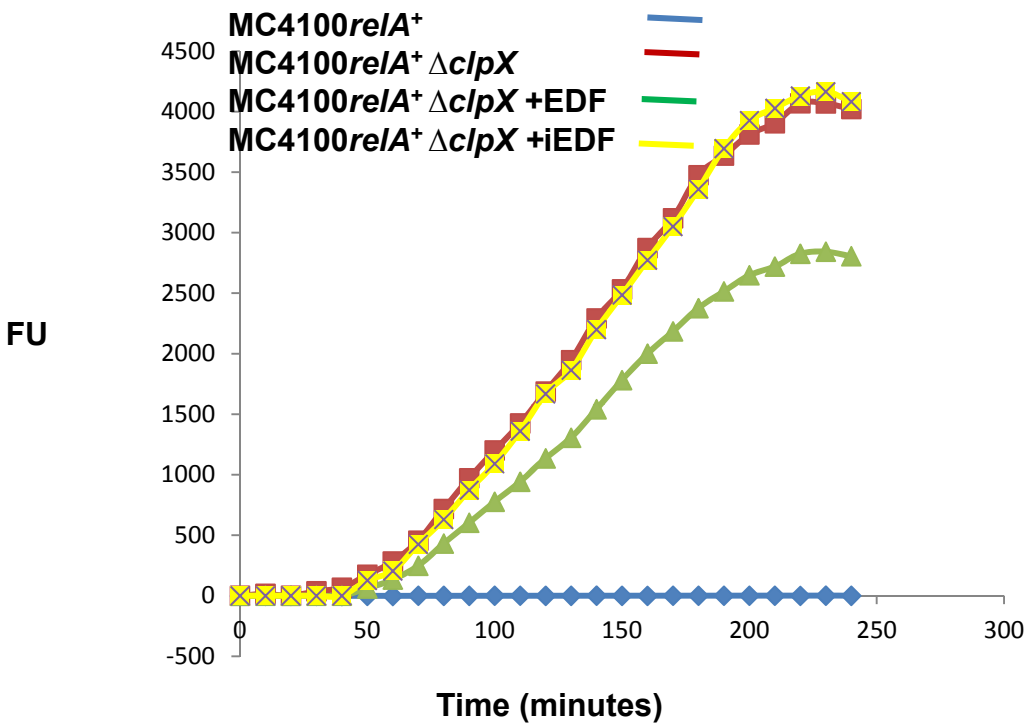
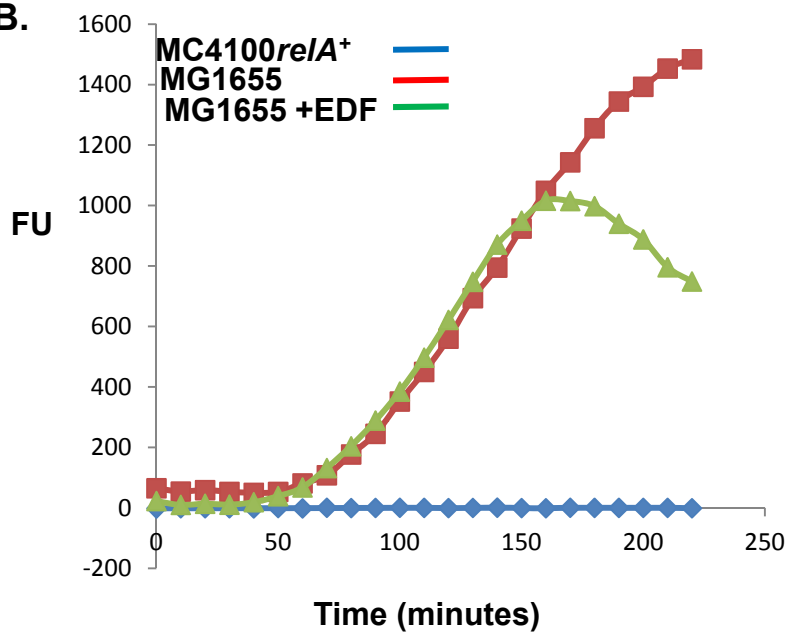

MC4100relA+
MC4100relA+ ΔygcR
FU
4000
3500
3000
2500
2000
1500
1000
500
0
-500
0
50
100
150
200
250
Time (minutes)

Figure 2. **EDF is involved in the inhibition of the SOS response by the *mazEF* pathway.** *Escherichia coli* strains were studied: MC4100*relA*⁺Δ*clpX* (A), MG1655 (B), and BW25113.

A.



B.


MC4100relA+
MG1655
MG1655 +EDF
FU
1600
1400
1200
1000
800
600
400
200
0
-200
0
50
100
150
200
250
Time (minutes)

C.

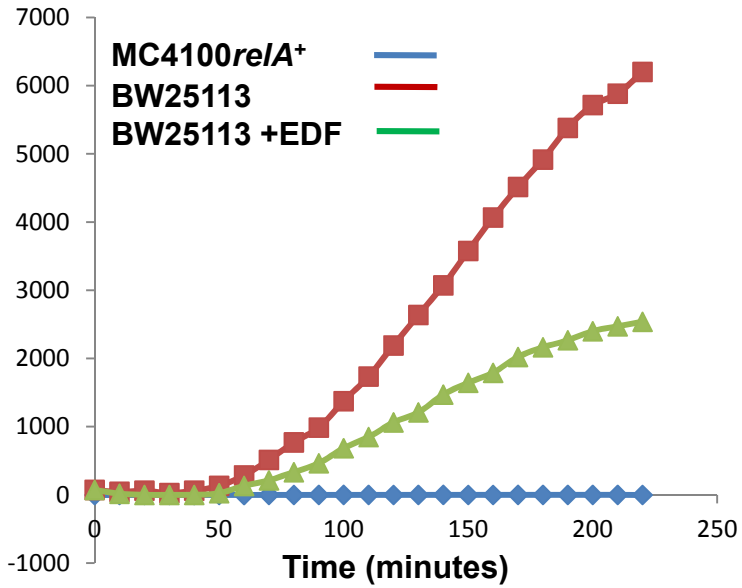
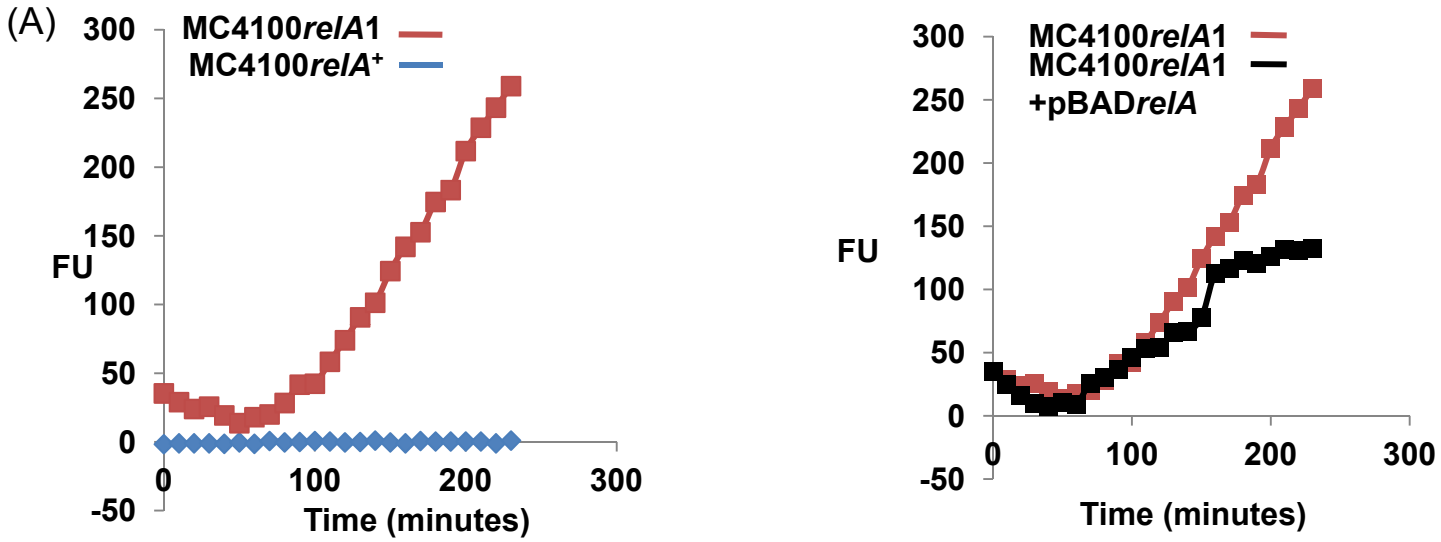
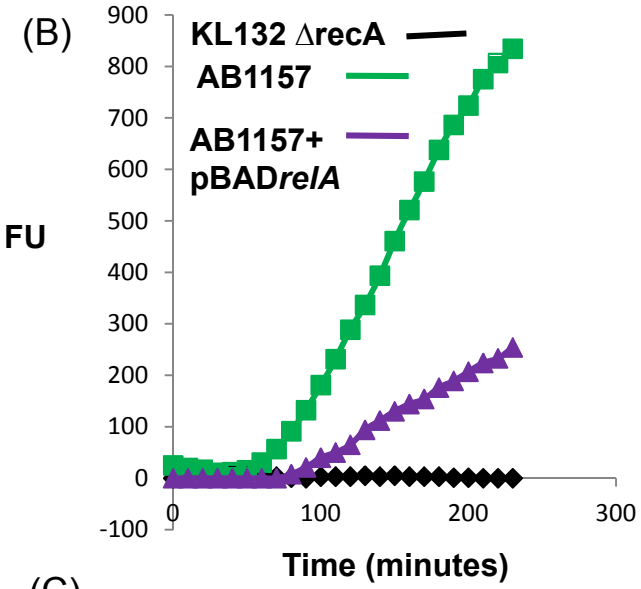
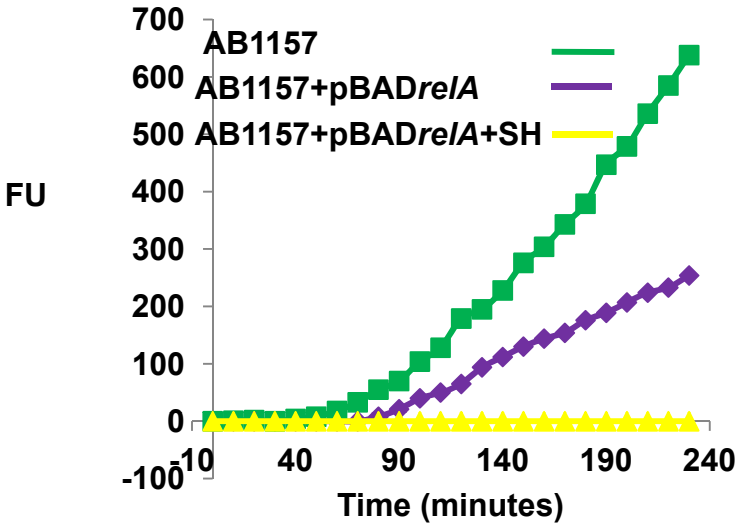

MC4100relA+
BW25113
BW25113 +EDF
FU
7000
6000
5000
4000
3000
2000
1000
0
-1000
0
50
100
150
200
250
Time (minutes)

Figure 3 **The SOS response is prevented in *Escheichia coli* strains carry a functional *relA*$^{+}$ gene.**



(B)


KL132 ΔrecA
AB1157
AB1157+
pBADrelA
FU
900
800
700
600
500
400
300
200
100
0
-100
0
100
200
300
Time (minutes)


AB1157
AB1157+pBADrelA
AB1157+pBADrelA+SH
FU
700
600
500
400
300
200
100
0
-100
-10
40
90
140
190
240
Time (minutes)

(C)

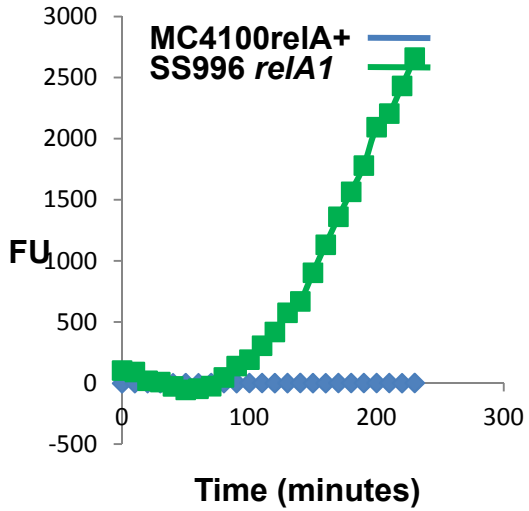

MC4100relA+
SS996 relA1
FU
3000
2500
2000
1500
1000
500
0
-500
0
100
200
300
Time (minutes)

Figure 4

ƛ lysogen overcomes the inhibitory effect of *mazEF* on the SOS response.

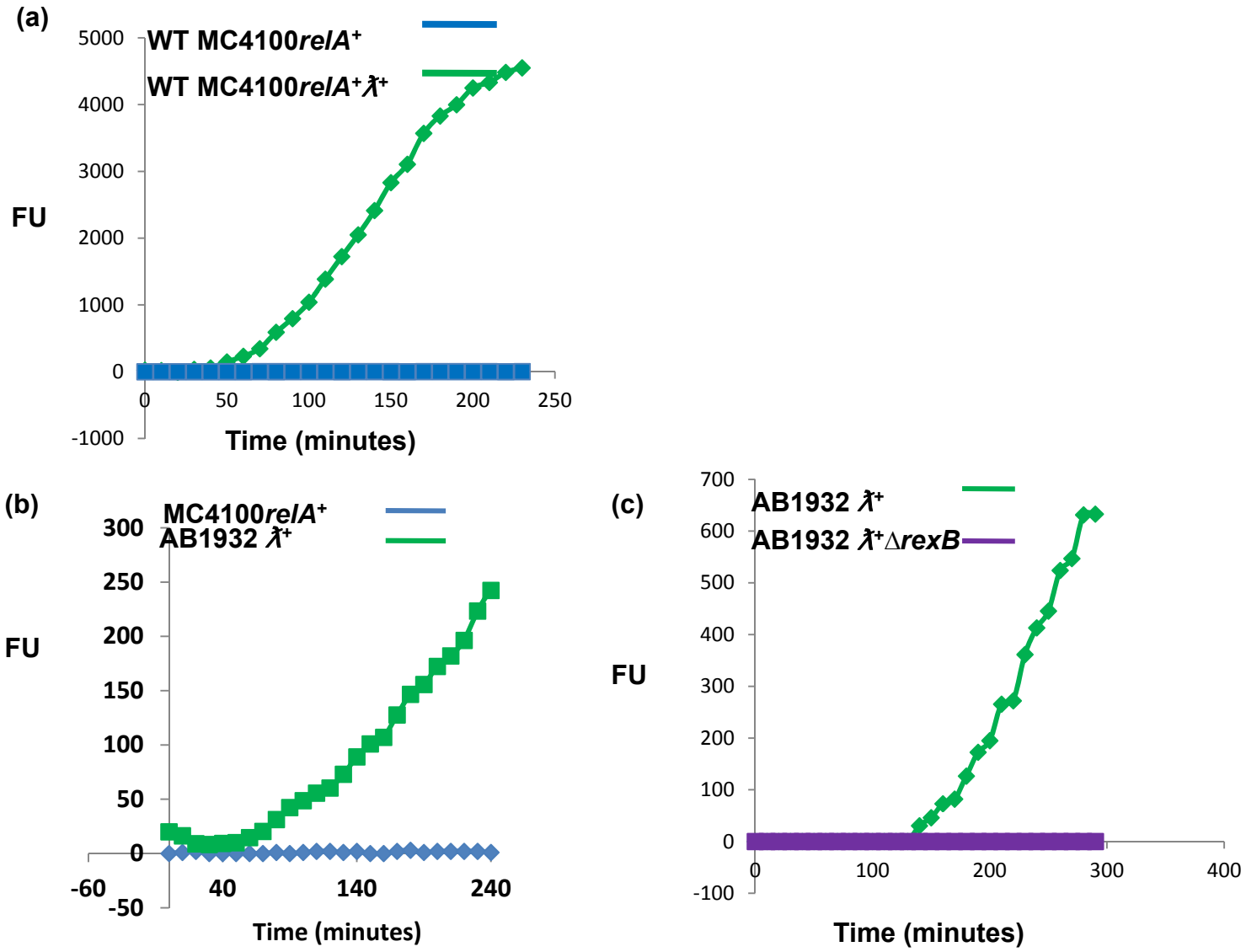


Table 1
The Identified elements related to the EDF-*mazEF* pathway that permitted the SOS response in commonly used *Escherichia coli* strains.

strain	The studied element		
	ppGpp production	EDF	ƛ lysogen
MG1655	+	-	-
BW25113	+	-	-
MC4100*relA1*	-	+	-
AB1157	-	+	-
SS996	-	+	-
AB1932	+	+	+